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A DNA prime/protein boost vaccination trial was initiated against challenge with a chimeric simian-human immunodeficiency virus, termed SHIV-vpu$^+$, in newborn macaques. DNA vectors coding for *gag*, *pol* and *nef* genes of SIVmac239 and for HIV-IIIB *env* were used to prime followed by boosting with recombinant, homologous HIV-1 gp160. Following challenge with nonpathogenic, homologous SHIV-vpu$^+$, partial protection (as defined by no or low, transient cytoviremia) was observed in 20% of the animals that received DNA priming/protein boost vaccination and in 3 of 4 animals given the gp160 boosts only. These partially protected animals had high neutralizing antibody titers at time of challenge. Six completely or partially protected animals were rechallenged 4 months later with homologous virus. All showed evidence of either rapid viral clearance or low viral loads, suggesting that protective immunity was long-lived. The same 6 vaccinated animals were rechallenged a third time with heterologous, pathogenic SHIV89.6P. Four of these 5 animals maintained normal CD4$^+$ T-cell counts and showed evidence of limited virus infection. Neutralizing antibody titers, T-cell proliferation and T-cytolytic T lymphocyte responses offer evidence that both humoral and cellular immune mechanism contributed to the observed protection against pathogenic virus challenge. Our results indicate that DNA priming alone can induce antibody responses in newborn macaques and that protein boosts can induce some degree of protective immunity against viral challenge with both homologous and heterologous SHIV.

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Ruth M. Reynolds 3/24/00

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ABSTRACT

Augmentation of naked DNA immunization by boosting with soluble proteins, recombinant fowl pox or vaccinia viruses has been documented as a means to induce both humoral and cellular protective immune responses against immunodeficiency virus challenge. In this study, a DNA prime/protein boost vaccination trial was initiated against challenge with a chimeric simian-human immunodeficiency virus, termed SHIV-vpu$^+$, in newborn macaques. DNA vectors coding for *gag*, *pol* and *nef* genes of SIVmac239 and for HIV-IIIB *env* were used to prime followed by boosting with recombinant, homologous HIV-1 gp160. Following challenge with nonpathogenic, homologous SHIV-vpu$^+$, partial protection (as defined by no or low, transient cytoviremia) was observed in 20% of the animals that received DNA priming/protein boost vaccination and in 3 of 4 animals given the gp160 boosts only. These partially protected animals had high neutralizing antibody titers at time of challenge. Six completely or partially protected animals were rechallenged 4 months later with homologous virus. All showed evidence of either rapid viral clearance or low viral loads, suggesting that protective immunity was long-lived. The same 6 vaccinated animals were rechallenged a third time with heterologous, pathogenic SHIV89.6P. Four of these 5 animals maintained normal CD4$^+$ T-cell counts and showed evidence of limited virus infection. Neutralizing antibody titers, T-cell proliferation and T-cytolytic T lymphocyte responses offer evidence that both humoral and cellular immune mechanism contributed to the observed protection against pathogenic virus challenge.

Our results indicate that DNA priming alone can induce antibody responses in newborn macaques and that protein boosts can induce some degree of protective immunity against viral challenge with both homologous and heterologous SHIV.

INTRODUCTION

An estimated 1.2 million children are infected with HIV worldwide with 90% having become infected through maternal transmission. Globally, but particularly in sub-Saharan Africa where the expensive combination antiviral cocktail treatment for HIV-1 infection now used in industrialized countries is neither technically feasible nor economically possible, an inexpensive, safe, and effective vaccine to induce protective immunity would be the best solution. However, inherent problems for designing an effective vaccine include the natural antigenic diversity of HIV-1, infection by cell-free and cell-associated virus via mucosal routes, and a lack of consistent correlates of immune protection to dictate which arm of an immune response should be mobilized to a greater degree.

Of the number of approaches examined to generate protective immunity against HIV-1, including the use of live attenuated viruses and protein sub-unit vaccines, the use of nucleic acid immunization has received considerable attention in recent years. Using this approach, immune responses to antigens encoded by plasmid DNA have been raised in a number of infectious disease models (5, 13, 26, 41).

For generating immune protection against HIV-1 infection, DNA vaccination offers several advantages over more conventional vaccine approaches. First, the relative low cost to prepare and deliver DNA plasmids for third world endemic sites is appealing. Second, while the use of live attenuated virus vaccines to mimic natural virus infection and induce virus-specific immune responses, including cytotoxic T lymphocyte (CTL) responses, may eventually be possible, their current safety is questionable in light of their proven pathogenic potential (2, 3). Third, unlike sub-unit vaccines or whole inactivated virus, which induce predominantly humoral immune responses, DNA immunization evokes both humoral and cellular immunity, including CTL responses (14, 23, 24, 43, 44). The relative contribution of neutralizing antibodies (nAbs) and MHC-restricted CTL responses to overall immune protection against HIV-1 infection is not clear at this point. However, it is generally considered from evidence of HIV-1 exposed, long-term nonprogessors or uninfected individuals, that generating both would be most beneficial (7, 10, 20, 38).

In one study demonstrating the potential for DNA vaccination, chimpanzees inoculated with a series of DNA plasmids encoding HIV-1 *env*, *rev* and *gag/pol* showed antibody responses to HIV-1 antigens and had variable levels of CTL activity (9). Following challenge with heterologous HIV-1, the inoculated animals showed strong evidence of protection from infection as measured by RT-PCR analysis. However, in separate studies, DNA vaccines alone showed only limited protection from pathogenic SIV or non-pathogenic $SHIV_{HXB2}$ infection of macaques despite strong cellular immune responses (8, 25). In a recent study, though, the potential of DNA vaccination for preventing mother-infant transmission was demonstrated; intravaginal and intramuscular (im) DNA vaccination of pregnant chimpanzees with HIV-1 *env/rev* and *gag/pol* encoding plasmids induced Gag-specific cellular immune responses, IgA responses in milk, and placental transfer of anti-Env and anti-Gag IgG (6).

Studies designed to augment the immune responses evoked from DNA immunization have recently been evaluated. They are based on the idea of a dual modality of immunization, instead of a singular DNA-based immunization scheme that alone may induce a strong CTL response at the expense of humoral immunity or a more broad-based T-cell response. In one prime/boost system consisting of gene-gun inoculation of juvenile macaques with Env and Gag-expressing DNA followed by boosting with recombinant fowlpox virus, HIV-1-specific CTL and helper T-cell responses increased over DNA vaccination alone (17). No difference in antibody titers after boosting was noted. Vaccinated macaques were subsequently protected from intravenous (iv) HIV-1 challenge. In a separate study, Letvin et al. were able to augment the strong CTL response seen following DNA vaccination alone with HIV-IIIB *env* expressing plasmids, by boosting macaques with Env protein (21). A strong increase in nAb titer was seen with only a modest increase in CTL activity. Complete protection following i.v. challenge with SHIV-HxBc2 was seen in 2 of 2 animals given this prime/boost regimen.

In a similar manner, Robinson et al. used a combined DNA prime/protein or recombinant fowl pox boost regimen in a SHIV-IIIB challenge system in adult macaques (37). The greatest protection was seen in with intradermal priming followed by recombinant fowl pox boosting. Protection was apparently not dependent on nAbs.

In this study, a cohort of neonatal macaques was given a series of DNA priming inoculations using plasmids encoding SHIV-vpu$^+$ (also called SHIV-IIIB) antigens followed by booster inoculations with soluble HIV-IIIB gp160. Protection against homologous, nonpathogenic SHIV-IIIB challenge was observed in 20% of the animals and in 3 of 4 animals that received protein boosts only. Rechallenge experiments showed this protection was long-lived and, for most protected animals, broad-based, offering protection against heterologous, pathogenic SHIV89.6P challenge.

Publications since the report of November, 1998:

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Liska V, Ruprecht RM. Isolation of high-molecular-weight genomic DNA from intact hazardous mammalian tissues. *BioTechniques* 1999; 26:62-6.

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Rasmussen RA, Hofmann-Lehmann, Li P-L, Schmitz JE, Reimann KA, Montefiori DC, McClure HM, Ruprecht RM. $CD8^+$ T cells and neutralizing antibodies control viremia and disease in SHIV-infected macaques. In preparation.

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MATERIALS AND METHODS

DNA constructs, protein immunogen and virus stocks. The following DNA plasmids were used for the DNA priming phase of the vaccination protocol: 1) pRS102, encoding the Gag and polymerase proteins of SIVmac239, 2) pCMV/nef, encoding SIVmac239 *nef*, 3) pJW4303/HXB-2 pol, a construct designed to allow expression of non-infectious HIV1-HXB-2 particles, 4) pJW4303/HXB-2.gp140 allowing expression of the gp120 subunit of HXB-2 Env, and 5) pJW4303/HXb-2.gp120 encoding a secreted monomeric form of gp120 of HIV-IIIB (35, 37). Recombinant gp160 was prepared from a monkey cell line infected with vaccinia virus expressing full-length, nonmutated HIV-IIIB *env* (32). SHIV-vpu$^+$, which contains the *tat, rev, vpu*, and *env* genes from HIV-IIIB, was used for initial virus challenges (4) and SHIV89.6P was kindly provided by Dr. Keith Reimann (16, 34).

Animals and immunization. DNA was given either by intradermal (id) injection or gene-gun (gg) inoculation and consisted of a total of 4 inoculations at the time intervals shown in Figure 1. The total DNA dose per animal was 250 μg of each DNA vector for id route and 2 μg of each DNA vector for gene gun inoculation. In some groups, recombinant IL-12 (1.5 μg /kg body weight) was given subcutaneously (sq) at the time of each DNA inoculation. After animals were rested for 10 mos, each was boosted im 2 x at 4 mos intervals with 100 μg recombinant gp160 in incomplete Freunds adjuvant (animals that had been inoculated with non-*env* encoding DNA plasmids were reinoculated with the same DNA plasmids at each protein boost time point). Two weeks after the final boost, animals were challenged iv with 10 50% animal infectious doses (AID_{50}) of SHIV-vpu$^+$.

ELISA for antibody responses. Nunc Immuno plates (MaxiSorb F96) (Roskilde, Denmark) were coated with gp160 of HIV-IIIB (Quality Biologicals, Inc., Gaithersburg, MD) by adding 100 μl of a solution containing 1 μg protein/ml of carbonate buffer (15 mM Na_2CO_3, 35 mM $NaHCO_3$, pH 9.8) and incubating overnight at 4°C. The solution was aspirated, the wells washed once with 100 μl of phosphate-buffered saline (PBS; 0.14 M NaCl, 2.7 mM KCl, 8 mM Na_2HPO_4, 1.7 mM NaH_2PO_4, pH 7.4) and then filled with 100 μl of blocking buffer (95% v/v PBS, 5% w/v bovine serum albumin and 5% v/v growth medium) and incubated for 2 hr at 37°C. The plates were washed 4 x with PBS containing 0.05% v/v Tween 20. Serum samples were diluted 1:50 in borate

buffer (0.1 M boric acid, 47 mM sodium borate, 75 mM NaCl, 0.05% v/v Tween-20) containing 2.5% fetal bovine serum, added to the wells (each in duplicate) and incubated for 2 hr at 37°C. Wells were washed 4 x with PBS/Tween-20 and received 100 µl of alkaline-phosphatase-conjugated, goat anti-monkey IgG (whole molecule, Sigma Chemical Company, St. Louis, MO) and incubated for 2 hr at 37°C. The wells were washed 4 x with PBS/Tween-20 and incubated with 100 µl of p-nitrophenylphosphate disodium hexahydrate (Sigma 104 phosphatase substrate, Sigma) in diethanolamine buffer (0.9 M diethanolamine, 7 mM $MgCl_2$, pH 9.8 with concentrated HCl). Following color development, the absorbency was read at 405 nm.

Neutralizing antibody assays. Antibody-mediated neutralization of SHIV-vpu$^+$ and SHIV-89.6P was assessed in an MT-2 cell killing assay as described previously (12, 27). Briefly, 50 µl of cell-free virus containing 500 50% tissue-culture infectious doses ($TCID_{50}$) were added to multiple dilutions of test serum in 100 µl of growth medium (RPMI-1640 containing 12% fetal bovine serum and 50 µg gentamicin/ml) in triplicate in 96-well culture plates. The mixtures were incubated for 1 hr at 37°C followed by the addition of MT-2 cells (5 x 10^4 cells in 100 µl) to each well. Infection led to extensive syncytium formation and virus-induced cell killing in approximately 4 - 6 days in the absence of antibodies. Neutralization was measured by staining viable cells with Finter's neutral red in poly-L-lysine-coated plates as described (27). Percent protection was determined by calculating the difference in absorption (A_{540}) between test wells (cells + serum sample + virus) and virus control wells (cells + virus), dividing this result by the difference in absorption between cell control wells (cells only) and virus control wells, and multiplying by 100. Neutralization was measured at a time when virus-induced cell-killing in virus control wells was greater than 70% but less than 100%. Neutralization titers are given as the reciprocal dilution required to protect 50% of cells from virus-induced killing. Cell-free stocks of SHIV-vpu$^+$ and SHIV-89.6P were prepared in H9 cells and human peripheral blood mononuclear cells (PBMC), respectively (12, 28).

CTL activity. Autologous B lymphocytic cell lines (BLCL) were prepared for each enrolled animal by transformation of PBMC with herpes virus papio (33). PBMC were stimulated with paraformaldehyde-fixed, autologous BLCL infected with SHIV antigen-encoding vaccinia virus constructs at 2 - 3 PFU/cell (Therion, Cambridge, MA). Recombinant human Il-2 (20 U/ml) was added every 3 days. Cultures were tested on day 7 in a standard 5 hr chromium release assay for CTL activity against autologous ^{51}Cr-labeled BLCL target cells infected with vaccinia constructs encoding SIV *gag-pol*, SIV *nef*, or HIV-IIIB *env.* Background killing was measured by using control target cells infected with wild-type vaccinia virus. Background cytotoxicity has been subtracted when % specific lysis is shown. In all assays, a culture of PBMC from an animal with known CTL activity was included as a positive control for the stimulation and cytotoxicity arms of the assay. PBMC from an SIVmac251-infected animal with known CTL activity were always included in each assay as a positive control.

Virus Cocultivation Assays. PBMC were purified from heparinized whole blood by Ficoll density gradient centrifugation or by centrifugation in CPT tubes (Becton-Dickinson, Franklin Lakes, NJ). PBMC were serially diluted and cultured in the presence of CEMx174/GFP cells, a T-cell line expressing Green Fluorescent Protein under transcriptional control of an HIV-2 long terminal repeat element (cells kindly

provided by Dr. Barbara Felber, NCI). Cocultivation media, consisting of RPMI-1640, 15% FCS, L-glutamine and penicillin/streptomycin, was replaced every 3-4 days. After 21 days, wells were scored for fluorescent cell positive wells and culture supernatants collected for determination of p27 Gag antigen levels using a commercial ELISA kit (Coulter, Miami, FL).

DNA PCR analyses: High molecular weight genomic DNA was isolated from frozen monkey PBMC using DNAzol® (Molecular Research Center, Cincinnati, OH) under the manufacturer's recommended conditions.

Differential SHIV-vpu$^+$/SHIV89.6P DNA PCR assay. In order to distinguish between SHIV-vpu$^+$ and SHIV89.6P proviral DNA, specific oligonucleotides, spanning the 140-bp deletion in *env* gene of SHIV89.6P (16), were employed. One g of genomic PBMC DNA was amplified with primers 8,329 to 8,353 (5'-GGCAAGTTTGTGGAATTGGTTTGA-3') and 8,903 to 8,926 (5'-CCTTGTCTAATCCTCCTGGGGATT-3') (GeneBank; Accession number: U89134) in 10 mM Tris-HCl (pH 9.2), 1.5 mM $MgCl_2$, 25 mM KCl (Opti–Prime buffer #9; Stratagene, La Jolla, CA). PCR reaction mixture also contained 50 picomoles of each primer, 100 moles of each dNTP and 1.25 U of *Taq* polymerase (AmpliTaq® DNA Polymerase; Roche Molecular Systems, Branchburg, NJ). The *Taq* polymerase was inactivated with *Taq*Start antibody (Clontech, Palo Alto, CA) according to the manufacturer's specified conditions. Cycling conditions were as follows: Initial denaturation (98°C, 15 s) followed by six cycles of "touchdown" amplification (97.5°C,15 s; 58°C, 40 s; 72°C, 55 s). Then, cycling continued (94°C, 15 s; 53°C, 15 s; 72°C, 30 s) for 40 cycles, and was finished at 72°C for 6 min. PCR products (10 µl of 50 µl total) were separated by electrophoresis through a 2.5% agarose gel and stained with ethidium bromide. Sensitivity of the assay was 1 proviral copy / 1 µg of genomic DNA (approximately equivalent to 150,000 cells). This qualitative DNA PCR assay allows detection of both proviral DNAs (SHIV-vpu$^+$ and SHIV89.6P) simultaneously in ratios ranging from 1:10 to 10:1 copies.

Diagnostic SHIV89.6P DNA PCR assay. In order to detect the SHIV89.6P provirus in monkey PBMC DNA samples containing high numbers of SHIV-vpu$^+$ proviral copies, a SHIV89.6P specific DNA PCR assay was employed. Genomic PBMC DNA (1.0 µg) was amplified with primers 8,164 to 8,195 (5'-ATGTTAGTTGGAGTAATAAATCTGTGGATGAT-3') and 8,918 to 8,948 (5'-TCACAAGAGAGTGAGCTCAAGCCCTTGTCT-3') in the same PCR reaction mixture as described above and using identical cycling conditions. Then, 1 µl of amplicons was amplified by second-round (nested) PCR with primers 8,546 – 8,575 (5'-AGAGAGAGACAGAGACAGATCCGGTCCATC-3') and 8,875 to 8,904 (5'-TTGGCAGTATCCATCTTCCACCTCTGCTAA-3') under the same PCR reaction mixture conditions as described for first-round PCR. Cycling conditions were as follows: Initial denaturation (98°C, 15 s) followed by six cycles of "touchdown" amplification (97°C,15 s; 72°C, 45 s; 72°C, 60 s). Cycling was then continued (94°C , 15 s; 63°C, 15 s; 72°C, 30 s) for 40 cycles and finished at 72°C for 6 min. PCR products (10 µl of 50 µl total) were separated by electrophoresis through a 2.5% agarose gel and stained with ethidium bromide. Sensitivity of the assay is 1 proviral copy / 1 µg of genomic DNA.

Quantification of viral RNA plasma load. Plasma viral load following the first virus challenge was determined by quantitative competitive (qc) RT-PCR as described (22). The PCR amplified a 336 bp fragment in the *gag* region of SIV. The lower limit of that assay for viral RNA given by the sample volume and the dilutions used was 2,721 copies/ml plasma. Samples collected later during the experiment were analyzed by quantitative real-time PCR (Hofmann-Lehmann et al., manuscript submitted). Briefly, RNA was extracted from 140 µl sodium citrate-anticoagulated plasma by the use of QIAamp Viral RNA Mini kit (Qiagen, Valencia, CA). RT-PCR was performed as a one-tube, one-enzyme assay with the TaqMan EZ RT-PCR kit (PE Biosystems, Foster City, CA) and run on a ABI Prism 7700 Sequence Detection System (PE Biosystems).

The conditions were as follows: the 50 µl mixtures contained 10 µl 5 x EZ TaqMan buffer, 2 mM $Mn(OAc)_2$, 300 µM dATP, dCTP, dGTP, 600 µM dUTP, 200 nM of each primer, 125 nM of the fluorogenic probe, 5 units of rTth DNA polymerase, and 1 unit of AmpErase uracil N-Glycosylase (UNG). Thermal cycling conditions consisted of 2 min at 50°C, 30 min at 60°C, 5 min at 95°C, followed by 50 cycles of 15 sec at 95°C and 1 min at 60°C.

The primers and probe used amplified a 92 bp fragment in the SIV gag region. The lower limit of detection of the assay was 100 RNA copies per ml plasma.

Antigen-Specific PBMC Proliferation. Triplicate cultures of PBMC were set up at 2 x 10^5 cells per well of flat-bottom, 96-well plates in RPMI-1640, 10% FCS, L-glutamine and pen/strep. Soluble SIVmac239 Gag, Nef, or HIV-1 LAV Env (all obtained through the AIDS Research and Reference Reagent Program, NIAID, NIH) were each included at 2 g/ml. After 4 days, wells were pulsed with 1µCi ^{3}H-TdR (NEN, Boston, MA) and cells harvested 18 hr later. Thymidine uptake was measured in a Betaplate Reader (Wallac, Gaithersberg, MD). Stimulation indices (SI) were determined by dividing the mean cpm from antigen or Con A-stimulated cultures by the mean cpm from cells cultured in medium alone.

RESULTS

Neonatal vaccination schema. A total of 5 groups of neonatal macaques, 4 animals per group, were vaccinated. The experimental groups and schedule of inoculations are shown in Table 1 and Figure 1. Vaccination was begun within hours of the birth of each animal. Each DNA inoculation consisted of a combination of 5 different DNA plasmid vectors per inoculation and was given either id (groups 1-3) or using a gene-gun (groups 4-5). For id inoculation, a total of 250 µg of each DNA plasmid was inoculated. For animals that were inoculated using a gene-gun, 2 µg of total DNA was injected. The direct delivery of DNA into antigen-presenting cells by DNA-coated gold particles via gene-gun inoculation portended the use of less DNA than was used for id DNA injection to generate immune responses (14, 30). A mixture of 5 plasmids was used that coded for: 1) SIVmac239 *gag* and *pol*, 2) SIVmac239 *nef*, 3) a construct designed to allow expression of non-infectious HIV-1 particles (strain HXBc2), 4) a construct encoding an unstable secreted oligomeric form of gp140 allowing expression of the gp120 sub-unit of HXB-2 Env, and 5) a construct encoding

a secreted monomeric form of HIV-IIIB Env (gp120) (24). Animals in groups 2 and 5 were also given recombinant IL-12 (1.5 μg/kg body weight) as an adjuvant at each time of DNA inoculation in an attempt to boost cellular immune responses to the DNA vaccination (42).

All of the DNA-vaccinated animals, except those in control group 3, were given 2 boosts with recombinant HIV-IIIB gp160 (32) at 10 mos and 14 mos after the final DNA vaccination.

Prechallenge immune responses. Before virus challenge, vaccinated animals were evaluated for humoral and cellular immune responses.

Humoral immunity of all animals was measured by ELISA to HIV-IIIB gp160 (Table 2). Two weeks after the third DNA inoculation, antibody responses were observed in 2 of 4 animals in Group 1 and in 1 of 3 animals tested in Group 2. Sera of animals in Groups 4 and 5 were not tested at this time. At about 10 months into the vaccination period, anti-Env antibodies were detected in 3 of 4 animals in Group 1, 2 of 4 animals in group 2, 1 of 3 in Group 4, and in 4 of 4 in Group 5. As expected, all animals in control group 3 were negative. No significant levels of neutralizing antibodies (nAbs) were found at this time in any of the vaccinated animals.

Three months after the last set of the 4 DNA inoculations, all of the animals were tested for any evidence of specific CTL responses against SIV Gag-Pol, SIV Nef, or to homologous HIV-IIIB Env. These experiments revealed no specific CTL responses in any of the vaccinated animals at this time point (data not shown).

Three weeks after the initial HIV-IIIB gp160 protein boost, all 4 animals of groups 1, 2, and 5, and 2 of 3 animals within Group 4 tested positive for anti-Env antibodies. Again, no animals in control group 3, which did not receive a gp160 boost, were positive for anti-Env antibodies. Neutralizing antibodies to SHIV-vpu$^+$ were detected in some animals and the reciprocal serum activity titers are also shown in Table 2. Two of 4 animals in each of groups 1, 2 and 5, and 1 of 3 animals in group 4, also had nAbs.

At the time of virus challenge, all animals were Western blot negative for anti-SIV Gag antibodies (tested on HIV-2 strips with known Gag crossreactivity (4) (data not shown). Neutralizing antibody responses were again tested in the vaccinated animals (Table 3). All animals in group 1, 3 of 4 animals each in groups 5 and 6, and 2 of 4 animals in group 2 had nAbs against homologous SHIV-vpu$^+$. One of 3 animals in group 4, RDw-5, had nAbs while the nAb titer of a second animal in the same group, RBw-5, dropped to below detectable levels. Neutralizing antibody titers were strong in many cases, particularly in 3 animals that received gp160 boosts only. One animal, RFw-5, vaccinated with DNA by gene-gun inoculation plus IL-12 adjuvant and boosted with gp160, had a tremendous nAb titer of 2,010.

Virus challenge. The vaccinated animals were challenged with homologous SHIV-vpu$^+$ (10 AID_{50} i.v.) two weeks after the second gp160 boost, and viral load in the animals

was examined in PBMC by cocultivation assays (Table 3). Culture supernatants were analyzed for the level of SIV p27 Gag antigen after 3 weeks of cocultivation and are expressed as the number of infectious cells per 10^6 PBMC. All control animals that were given repeated inoculations of vector backbone only (group 3) as well as the 4 naive animals (group 7) were virus-isolation positive at each time point. In contrast, no infectious cells were found in 1 of 4 animals of both groups 5 (animal RFw-5) and 6 (animal RTs-5) at any of the time points. In addition, 1 animal from group 2 (RMw-5), 1 from group 4 (RDw-5) and an additional 2 animals from group 6 (RGt-5 and RDt-5) were only transiently virus-isolation positive with low numbers of infectious cells. Such a temporal pattern of negative virus isolation is a strong indication of protection (37). Moreover, these protected animals were virus isolation negative by co-cultivation at weeks 9 and 11 post challenge. While all protected animals had nAb titers of >41, other animals with higher titers were not protected. It should be noted, however, that sterilizing immunity was not observed in any of the animals. Plasma samples tested by RT-PCR for viral RNA were all positive at week 4 post-challenge (data not shown).

The six animals which were either virus negative by virus cocultivation or only transiently positive at low levels after initial virus challenge were rechallenged 14 weeks later with 10 AID_{50} i.v. of the same SHIV-vpu$^+$ stock. These included one animal each from groups 2, 4, and 5 and 3 animals which were immunized solely with gp160 (animals are marked in bold in Table 3). The viral load in PBMC of the animals after rechallenge was measured by cocultivation and DNA PCR (Table 4). Three of the animals showed no evidence of virus at any time after rechallenge, while one had a transient infection and was positive for proviral DNA at week 2. The other two animals, RDw-5 and RDt-5, were positive for infectious virus but displayed very low viral loads. In contrast, viral loads in all 4 animals of a group of untreated, age-matched control animals inoculated with virus reached $>1{,}000/10^6$ PBMC by week 2. These results suggest that the protection initially observed in the vaccinated animals persisted over several months.

Challenge with heterologous, pathogenic SHIV89.6P. Eight weeks after the rechallenge with homologous, nonpathogenic SHIV-vpu$^+$, the same 6 animals were rechallenged with SHIV89.6P to assess if protective immunity was broad-based. This virus is highly pathogenic in rhesus macaques and causes rapid loss of CD4$^+$ T cells in a matter of weeks (16, 34). The CD4$^+$ T-cell counts of 4 of the 6 experimental animals stayed within normal limits (Figure 2), and those of animal RGt-5 dipped but then recovered to levels above 300 cells/mm^3. All 4 virus-only control animals challenged with SHIV89.6P had precipitous drops in their CD4$^+$ T-cell counts.

The viral load measurements in PBMC from the animals are shown in Table 5. Each of the 4 control animals had high virus loads by week 1 post-challenge that were maintained through week 12. In contrast, the 4 animals from the experimental, immunized cohort that maintained normal CD4$^+$ T-cell levels had transient peaks of viremia which dropped by week 8. The animal with the transient decrease in CD4$^+$ T-cell counts, RGt-5, had a high initial viral load that dropped to low levels by week 8. Plasma viral RNA levels measured by RT-PCR in these animals (Figure 3) correlated with the results of the cocultivation. Peak titers in all animals occurred at 2 weeks post-challenge. RDw-5 and RDt-5 maintained very low or undetectable plasma RNA levels throughout the challenge period. RFw-5 had a peak viremia level similar to the

controls, and RMw-5 had a 2 log lower peak viremia level but then viral RNA levels in both animals declined rapidly until they were also undetectable or very low. The plasma viral RNA levels in the 4 control animals rose sharply and then remained high throughout the 24-week assay period.

Because the vaccinated animals had previously been exposed to the non-pathogenic SHIV-vpu$^+$, identification of the viral RNA species during peak viremia was of critical importance. Comparative DNA PCR analysis of PBMC from SHIV89.6P-challenged animals using nested primer pairs was able to detect both types of challenge viruses through common sequences in *env.* Surprisingly, only the presence of SHIV-vpu$^+$ was detected in all 6 vaccinees (Figure 4a), even in the 2 animals with severe CD4$^+$ T-cell losses. Thus, while SHIV-vpu$^+$ proviral DNA was not detected in PBMC following homologous rechallenge in animals RMw-5, RFw-5, RGt-5, and RTs-5, proviral DNA from this virus became detectable after challenge with SHIV89.6P. To test the sensitivity of the DNA PCR involving primers to sequences common to both SHIV strains, we varied the ratios of the 2 proviral DNAs. The PCR could only detect the minor species of sequences if they were present at ratios no less than 1:10. However, using primers specific for unique sequences of SHIV89.6P *env*, proviral DNA from the heterologous virus was found in 4 animals (RMw-5, RFw-5, RGt-5, and RTs-5) but not in RDw-5 and RDt-5 (Figure 4b). These latter two animals were completely protected from the pathogenic SHIV89.6P challenge. These results are consistent with the idea that the heterologous SHIV89.6P challenge induced a burst of SHIV-vpu$^+$ replication. The proviral copy numbers of SHIV-vpu$^+$, which was already present in the animals at the time of the SHIV89.6P challenge, exceeded those of the incoming, pathogenic virus at least 10-fold.

Immune measurements after SHIV89.6P challenge. Neutralizing antibody titers and, when adequate numbers of PBMC were available, T-cell proliferation and CTL responses were measured after challenge with pathogenic SHIV89.6P (Table 6). Neutralizing antibody titers against SHIV89.6P in the 2 disease-free animals, RMw-5 and RFw-5, and in RGt-5 which had a transient drop in CD4$^+$ T cells, were all high (5,000-12,500 at week 12 post-challenge), while no nAbs were detected in the other 3 animals. Interestingly, Gag-specific CTL activity was now detected in RDw-5 and RDt-5, the other 2 animals which maintained normal CD4$^+$ T-cell counts and showed no evidence of SHIV89.6P by DNA PCR analysis. Significant T-cell proliferative responses against SIV Gag were also seen at 2 time points in both RDw-5 and RDt-5, suggesting that cellular immunity contributed to the observed protection of these animals from pathogenic SHIV89.6P challenge.

DISCUSSION

The aim of this study was to evaluate a combination DNA prime/protein boost immunization protocol in neonatal macaques as a means to induce protective immunity against SHIV challenge. Overall, the study consisted of two phases. The first phase involved the DNA prime/protein boost vaccination and subsequent measurements of the immune responses and protection following homologous SHIV-vpu$^+$ challenge. The second phase consisted of homologous and heterologous SHIV rechallenge of a subset of the originally vaccinated animals that had resisted the first challenge. In

summary, we found that 1) DNA vaccines are safe and immunogenic in newborn macaques; 2) four DNA inoculations alone induced anti-gp120 antibody responses in 10 of 15 vaccinated infants but did not induce nAb responses or significant CTL responses; 3) boosting with gp160 induced nAbs in 10 of 15 DNA vaccinated animals and in 3 of 4 age-matched vaccinees that only received gp160; 4) 20% of the animals given DNA prime/protein boosts and 3 of 4 given gp160 alone showed protection from SHIV-vpu$^+$ challenge as measured by cocultivation of PBMC - these animals had relatively high nAb titers but the presence of nAbs was not predictive of protection; and 5) the observed protection was long-lived and was broad-based in most cases.

One important finding from the first phase of this study was the level of immune protection in animals primed with DNA compared with protection in animals receiving only the gp160 boost. All DNA-vaccinated animals were boosted with soluble gp160 and, following challenge with SHIV-vpu$^+$, 20% showed evidence of immune protection. However, 3 of 4 age-matched animals that received gp160 alone were also protected. This finding suggests that DNA vaccination of the animals as neonates offered no advantage over protein boosts alone with gp160. This result is surprising in 2 respects. First, the low numbers of protected animals in the DNA primed and gp160 boosted groups differs from studies that showed protection from lentivirus challenge in monkeys using similar, dual modes of vaccination (17, 21, 32). Second, the protection from gp160 alone was unexpected based on earlier findings that showed no protection in macaques against SHIVHxBc2 challenge with gp160 subunit inoculation alone (32). Neonatal tolerance to the antigens encoded by the DNA plasmids was one possibility to account for the low numbers of protected animals in our study (40). However, the induction of anti-Env IgG in 10 of 15 animals after DNA vaccination alone suggests that complete neonatal tolerance did not occur. Nevertheless, one explanation of the results following the initial SHIV-vpu$^+$ challenge may involve a hidden degree of neonatal immunologic tolerance. In the group that received gp160 inoculations alone, it is possible that anti-Env humoral (and possibly, cellular) immunity kept viral replication at low enough levels to allow core antigen-specific cellular immune responses to arise quickly and clear the virus. In contrast, tolerance to immunodominant epitopes of core antigens may have been induced in most of the DNA-primed animals. In these animals, the eventual clearance of the challenge virus through immune mechanisms was slower due to the need to mount immune responses to alternate, less reactive epitopes. Our inability to detect antigen-specific CTL responses after DNA immunization would be consistent with this hypothesis.

A direct comparison of our results with the findings of many other studies that employed prime/boost vaccination regimens in rhesus macaques is not possible due to numerous differences in the experimental protocols. These include age of the animals, routes of priming inoculation and choice of priming plasmid vectors or vaccinia virus, choice of boosting components, and differences in the route and challenge virus. However, a study comparable to the one presented here was recently completed in juvenile macaques employing a DNA prime followed by boosting with either gp160 or recombinant fowl pox virus (37). The DNA plasmids used for immunization were identical, and the gp160 protein in both studies was prepared from vaccinia virus constructs in the same manner (37). A number of observations were also similar between the two studies. First, CTL responses following DNA

immunization ranged from negligible to very low in the juveniles. Similarly, we detected no significant CTL activity following the 4 rounds of DNA immunization in the neonates. At the start of our study, we were aware of the disappointing CTL responses in the juveniles and so we inoculated the neonates in two of our groups with recombinant IL-12 at the time of DNA vaccination. IL-12 has been shown in many systems to direct immune responses towards a Th-1 type responses resulting in the induction of a stronger cellular immune response (42). Such augmentation with IL-12 was also seen when DNA was used for immunization (18). In our study, however, using IL-12 as an adjuvant induced neither increased CTL activity nor resulted in greater protection from SHIV challenge.

Although we saw no evidence of significant antigen-specific CTL activity, anti-Env antibodies were induced in 10 of 15 animals following DNA immunization. However, nAbs to Env were detected only after gp160 boosting. Despite high titers of nAb in some of the animals, this humoral response did not correlate completely with protection. In the first phase of this study, protection from persistent infection was associated with nAb titers of >41, but high titers were not predictive of protection upon challenge. Interestingly, protection in the similar DNA prime/protein or fowl pox boost study in juveniles was also not correlated with nAbs at time of challenge (37). Currently, it is thought that a combination of both humoral immunity and antigen-specific CTL is required for complete protection from lentiviral infection (7, 10, 15, 20, 38). However, the ability of nAbs alone to induce immune protection is also documented (31). It is, therefore, possible that the protection we observed was due solely to nAb activity. However, despite our inability to detect CTL activity, cellular immune mechanisms may have contributed.

The mechanism of protection following homologous rechallenge of 6 vaccinated animals is not clearly apparent. At the time of rechallenge, viral loads in PBMC from the initial SHIV-vpu$^+$ challenge were below detection limits. This suggests that viral interference resulting in cellular resistance to superinfection was not a major factor in the protection observed after homologous SHIV-vpu$^+$ rechallenge (11, 39). However, the strong degree of protection following this rechallenge - 4 of 6 animals with no detectable virus and the 2 others with transient, low viral loads as measured by cocultivation and DNA PCR analysis of PBMC - suggests that the initial SHIV-vpu$^+$ challenge boosted protective immunity. Our measurements of nAb titers indicate that any boosting of anti-Env humoral immunity from the initial challenge certainly did not occur in all animals. Titers from the time of initial challenge either rose or declined in no apparent pattern. In addition, as we observed following the initial virus challenge, there was no correlation between nAb titers and protection from homologous SHIV-vpu$^+$ rechallenge. Although viral loads in all the rechallenged animals were kept either very low or below detection levels, the 2 animals that were virus positive (RDw-5 and RDt-5) had contrasting nAb levels. One animal, RDt-5, had the highest nAb titer of the 6 rechallenged animals while the nAb levels in plasma of RDw-5 had dropped to below detection levels at time of rechallenge.

There was wide variability and complexity in protection from viral infection and pathogenicity following rechallenge of the same 6 animals with pathogenic SHIV89.6P. Initially, the pattern of protection we observed following this challenge appeared to be inversely related to the magnitude of the viral load in the animals following the initial

2 challenges. Thus, the 2 animals that were SHIV-vpu$^+$ negative by cocultivation and DNA PCR after both challenge inoculations (RFw-5 and RTs-5) had the highest viral loads after SHIV89.6P challenge. However, in only one of the animals did the CD4$^+$ T cells drop (RTs-5), while the other animal maintained normal CD4$^+$ T-cell levels. This suggests that the straightforward prevention from superinfection as the means to protect from SHIV89.6P challenge was not the protective mechanism involved.

The DNA PCR results after rechallenge with the pathogenic SHIV89.6P were surprising and added to the complexity of the results. In 4 of 6 animals, it appeared that this rechallenge caused a burst of replication of SHIV-vpu$^+$ that had been below detection limits before the rechallenge. The results indicate that, given the sensitivity of the DNA PCR reaction, the SHIV-vpu$^+$ levels of proviral DNA within PBMC of these animals exceeded those of SHIV89.6P by at least 10-fold. Antigen-specific proliferation and CTL measurements after challenge allowed us to better elucidate protective mechanisms in these animals. RDw-5 and RDt-5 showed no nAb responses against SHIV89.6P following challenge but did have CTL and proliferative responses to SIV Gag. DNA PCR analysis seem to indicate that these protective mechanisms prevented SHIV89.6P infection. Animals RFw-5 and RMw-5 started to mount nAb responses to SHIV89.6P epitopes, yet had no evidence of CTL activity at the limited E:T ratios we could test. Both animals also had evidence of some cellular immunity. Viral loads were high enough, however, to allow the animals to alter the humoral antibody response to generate nAb to SHIV89.6P Env epitopes. The burst of SHIV-vpu$^+$ proliferation may have also contributed to protection by preventing superinfection by SHIV89.6P of SHIV-vpu$^+$-infected cells.

It is worth noting that at time of SHIV89.6P challenge, animals with HIV-IIIB Env (homologous to SHIV-vpu$^+$) specific nAb activity showed no cross-reactive neutralizing antibody activity to SHIV89.6P Env determinants. However, by week 8 after SHIV89.6P challenge, 3 animals had nAb activity to this virus in their plasma. These results suggest that the DNA priming/protein boost regimen and subsequent homologous virus challenges, all entailing the HIV-IIIB form of Env, did not subject the animals to a form of original antigenic sin. Functionally, SHIV-vpu$^+$ employs the *env* region of the T-cell line adapted strain of HIV-IIIB, while the *env* region of SHIV89.6P dictates a dualtropic specificity (16, 34). It would appear that antigenic determinants unique to SHIV89.6P Env, including a 140 bp deletion in the encoding gene, are distinct enough from those of SHIVIIIB Env to allow maturation of the humoral response to closely related antigens. This finding is important in its implication that DNA immunization against *env*-encoding regions of HIV-1, especially in newborns, does not limit later immunity to differing strains of the virus.

Our study indicates that DNA priming of neonates is safe and is an effective means of generating humoral immune responses. The utility of prime/boost regimens for potential vaccination of newborns against HIV-1 infection was also seen in this study. Future experiments using codon-optimized plasmid vectors may induce stronger cellular immune responses in newborns from the DNA immunization arm of the bimodal vaccination and enhance protection levels above those seen in this study.

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Figure Legends

Figure 1. Neonatal macaque vaccination and SHIV challenge schedule.

Figure 2. $CD4^+$ T-lymphocyte counts in vaccinated animals following SHIV89.6P challenge. Aliquots of PBMC were monitored at indicated time points after SHIV89.6P challenge for the frequency of T-cell subsets by flow cytometry according to a protocol described previously (1). Values indicate the cell number per l whole blood.

Figure 3. Plasma viral RNA levels following challenge with SHIV89.6P. Plasma samples were tested for viral RNA at indicated time points after challenge with SHIV89.6P by RT-PCR. Analysis shows total viral RNA and does not discriminate between SHIV-vpu^+ and SHIV89.6P viral RNA. The lower limit of detection was 100 RNA copies/ml plasma.

Figure 4. DNA PCR for SHIV-vpu^+ and SHIV89.6P. DNA from PBMC isolated from indicated animals at weeks 1, 2, 3, 5, and 8 post rechallenge with SHIV-vpu+ and at weeks 1, 2, 4, 8, and 12 after challenge with SHIV89.6P was used for PCR analysis. **A** - DNA PCR for both viruses was performed with primers spanning a common sequence. The PCR product for the *env* sequence of SHIV-IIIB (homologous to SHIV-vpu^+) is 498 bp and the PCR product for the SHIV89.6P *env* sequence is revealed as 358 bp. Titration experiments indicated that the assay can reveal the presence of both viruses simultaneously for ratios ranging from 1:10 to 10:1. **B** – DNA PCR for SHIV89.6P only was performed. Controls show PCR reaction products from unimmunized control animals inoculated with SHIV-vpu^+ or with SHIV89.6P at 1, 2, and 4 weeks after inoculation and indicate the specificity of this PCR for a unique SHIV89.6P *env* sequence.

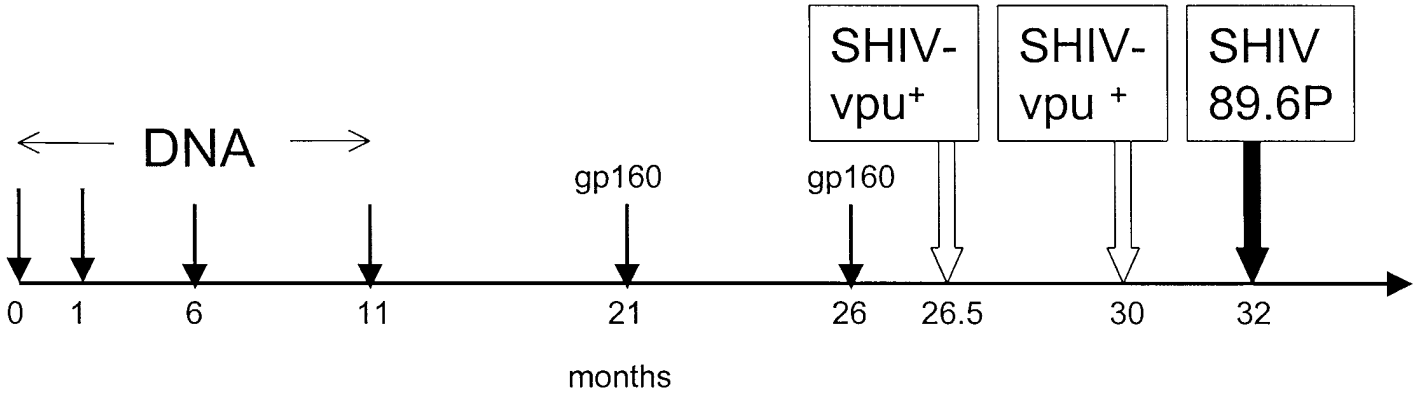

SHIV-
vpu+
SHIV-
vpu +
SHIV
89.6P
DNA
gp160
gp160
0
1
6
11
21
26
26.5
30
32
months

Fig. 1

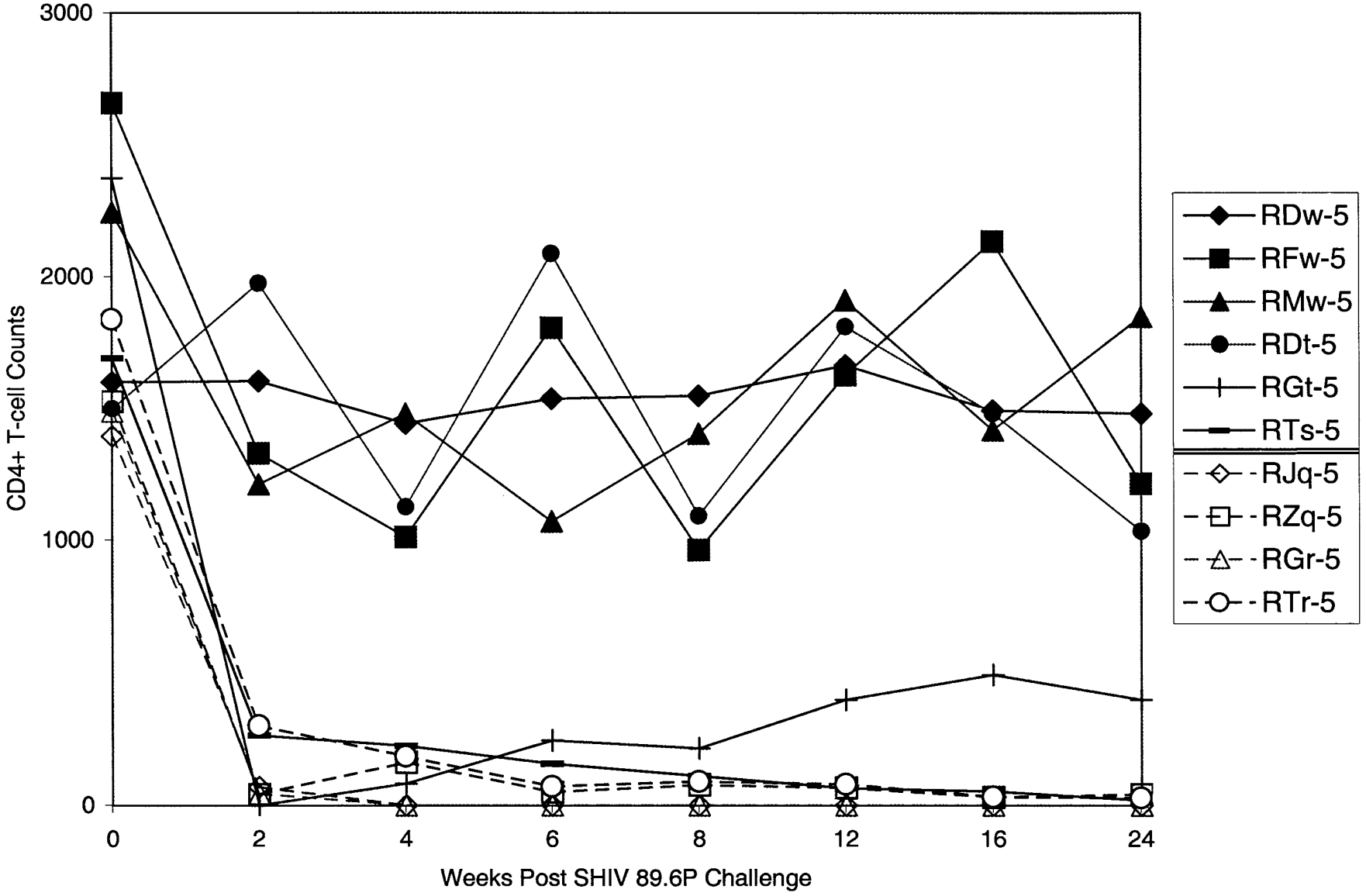

3000
2000
1000
0
CD4+ T-cell Counts
0
2
4
6
8
12
16
24
Weeks Post SHIV 89.6P Challenge
RDw-5
RFw-5
RMw-5
RDt-5
RGt-5
RTs-5
RJq-5
RZq-5
RGr-5
RTr-5

Figure 2

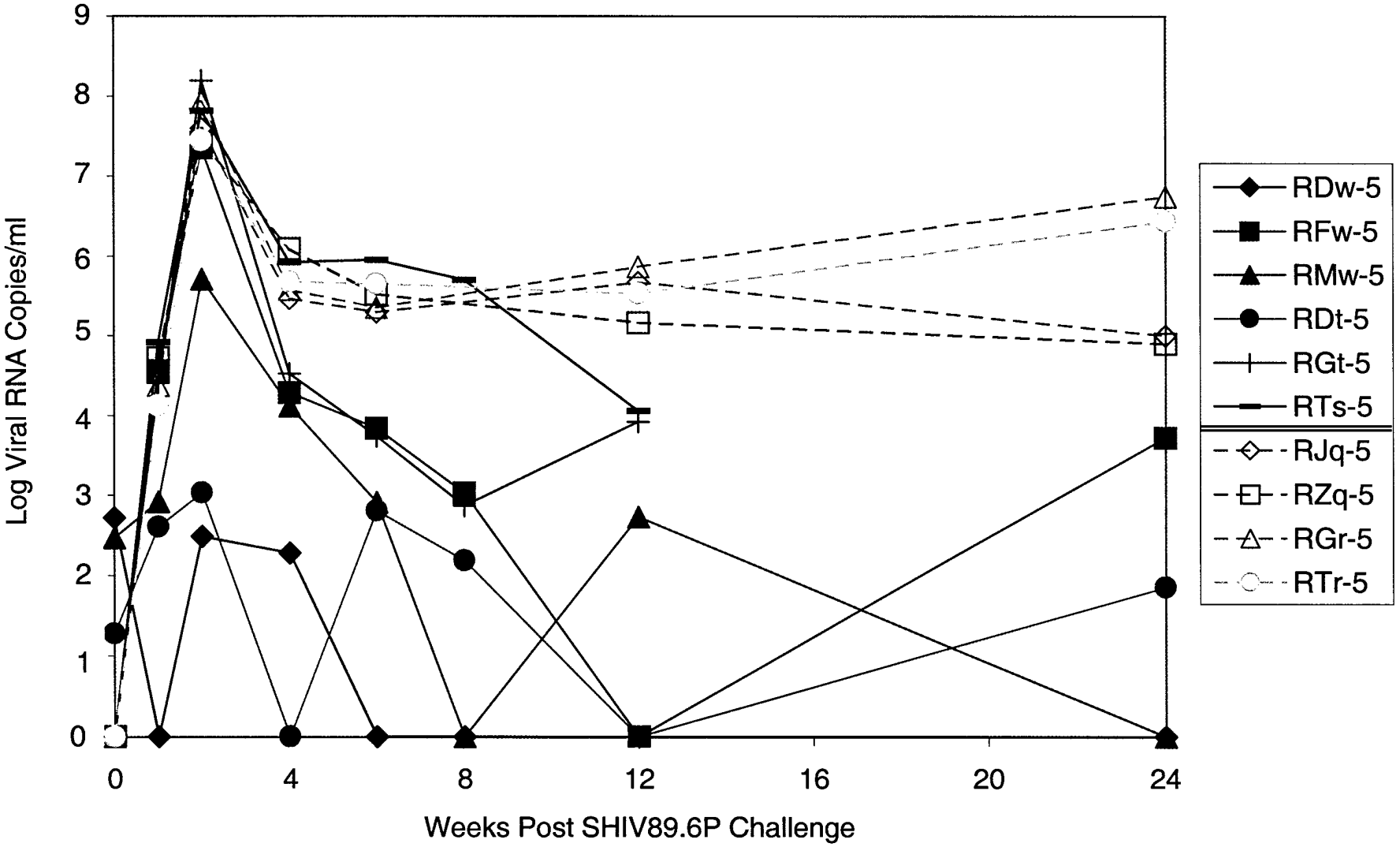
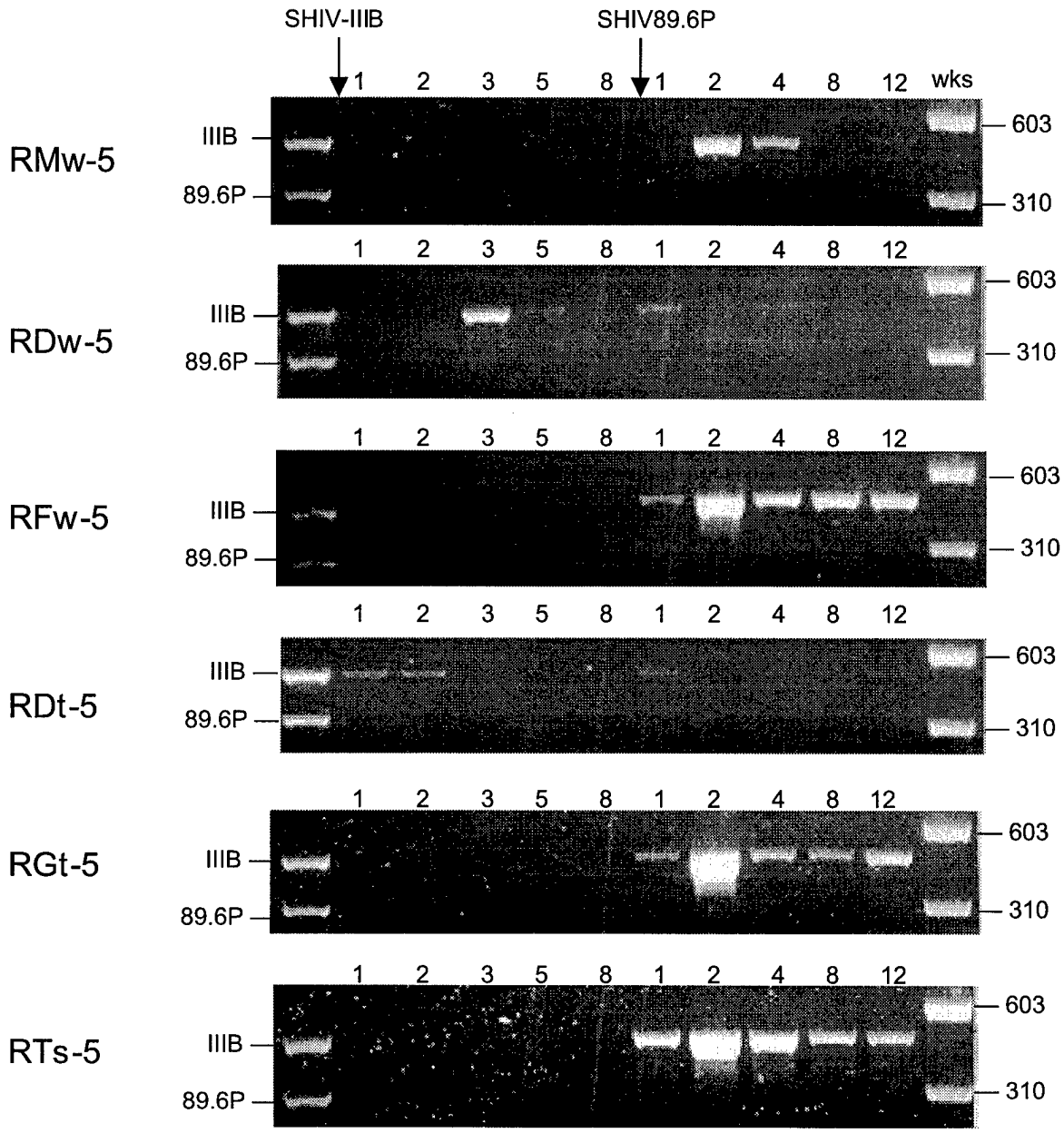

Log Viral RNA Copies/ml
9
8
7
6
5
4
3
2
1
0
0
4
8
12
16
20
24
Weeks Post SHIV89.6P Challenge
RDw-5
RFw-5
RMw-5
RDt-5
RGt-5
RTs-5
RJq-5
RZq-5
RGr-5
RTr-5

Figure 3

DNA PCR for SHIV-IIIB and SHIV89.6P



The assay can reveal presence of both viruses simultaneously for ratios ranging from 1:10 to 10:1.

Figure 4A

DNA PCR for SHIV89.6P only

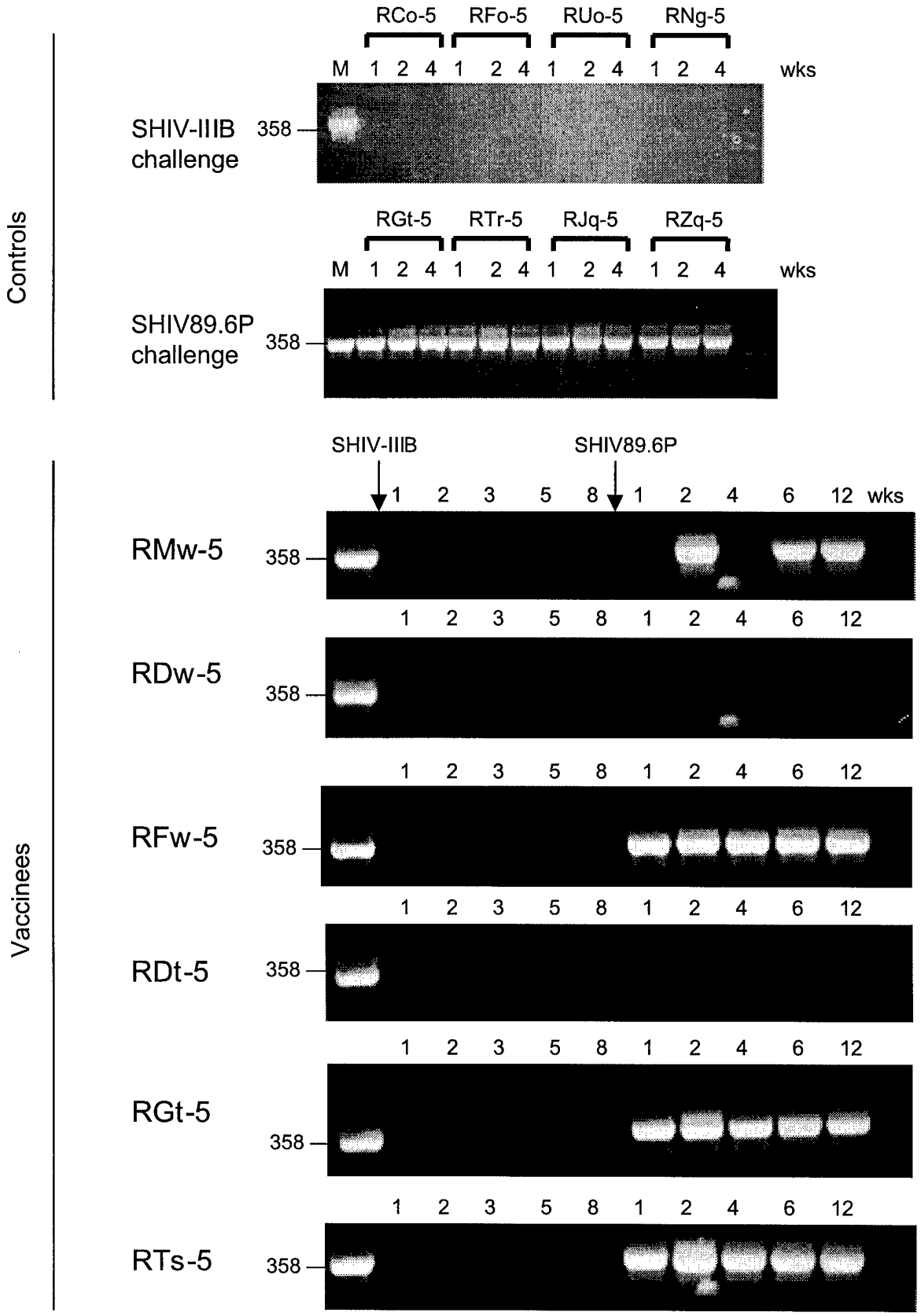


Figure 4B

TABLE 1. DNA priming/protein boost in neonatal macaques

Group #	N	DNA vaccination	Delivery	IL-12	Boost
1	4	5 DNAs	id	none	gp160
2	4	5 DNAs	id	+	gp160
3	4	Control	id	none	control DNA
4	3	5 DNAs	gene gun	none	gp160
5	4	5 DNAs	gene gun	+	gp160
6	4	none	-	-	gp160
7	4	none	-	-	none

TABLE 2. Pre-challenge antibody responses of neonatal macaques to DNA prime/gp160 boost inoculation.

			Month 7		Month 10.5		Month 22	
Group #	Animal #	DNA Vaccination	gp160 ELISA O.D. [1]	nAb [2]	gp160 ELISA O.D.	nAb	gp160 ELISA O.D.	nAb
1	REu-5	5 DNAs	2	<10	2.05	<20	1.42	24
1	RJu-5	(id)	0.72	<10	2.24	<20	1.38	<20
1	RIv-5		2.28	<10	3.1	<20	1.98	124
1	RUv-5		0.56	<10	0.48	<20	0.85	<20
2	RDv-5	id	0.24	<10	0.57	<20	0.70	<20
2	RTV-5	+ IL-12	0.29	<10	0.36	<20	0.90	<20
2	RYv-5		1.71	<10	1.78	<20	1.72	33
2	RMw-5		NT	<10	2.92	<20	2.11	32
3	RIu-5	Control DNA	0.2	<10	0.33	<20	0.22	<20
3	RWu-5		0.55	<10	0.45	<20	0.36	<20
3	RZu-5		0.33	<10	0.21	<20	0.25	<20
3	RVv-5		0.42	<10	0.2	<20	0.18	<20
4	RAw-5	Genegun	NT	NT	0.34	<20	0.38	<20
4	RBw-5		NT	NT	0.78	<20	0.98	32
4	RDw-5		NT	NT	0.38	<20	1.29	<20
5	RFw-5	Genegun	NT	NT	1.67	<20	1.78	<20
5	RGw-5	+ IL-12	NT	NT	2.1	<20	1.38	193
5	RIw-5		NT	NT	1.22	<20	1.56	<20
5	RJw-5		NT	NT	1.37	<20	1.19	29
6	RGt-5	gp160 alone					0.44	<20
6	RDt-5						0.52	<20
6	RNs-5						0.31	<20
6	RTs-5						0.68	<20

1 - Serum from inoculated animals was tested for antibodies to HIV-1-IIIB gp160 by ELISA.

2 – Reciprocal serum titers from inoculated animals with neutralizing activity against SHIV-HXB2.

TABLE 3. Neutralizing antibody levels and viral load upon challenge with SHIV-vpu^{+}.

Group #	Animal	DNA Vaccination	nAb Titer [1]	# infectious cells per 10^6 PBMC [2]				
				Week 2	Week 4	Week 6	Week 9	Week 11
1	REu-5	id	89	4	1024	16	1	<1
1	RJu-5		45	64	16	4	<1	1
1	RIv-5		174	1	1	16	4	<1
1	RUv-5		49	16	16	4	<1	1
2	RDv-5	id + IL-12	<20	256	16	64	4	4
2	RTV-5		47	64	4	16	4	<1
2	RYv-5		<20	64	4	4	<1	<1
2	RMw-5		41	4	<1	<1	<1	<1
3	RIu-5	Control DNA	<20	1024	1024	1024	4	16
3	RWu-5		<20	1024	64	16	16	1
3	RZu-5		<20	16	4	4	<1	1
3	RVv-5		<20	256	64	64	4	1
4	RAw-5	Genegun	<20	1024	4	2	4	<1
4	RBw-5		<20	64	16	8	<1	<1
4	RDw-5		138	<1	16	<0.5	<1	<1
5	RFw-5	Genegun	2010	0	<1	<0.5	<1	<1
5	RGw-5	+ IL-12	115	1	1	32	1	<1
5	RIw-5		51	16	1	32	<1	<1
5	RJw-5		<20	64	4	2	<1	<1
6	RGt-5	gp160 alone	410	16	<1	<0.5	<1	<1
6	RDt-5		145	<1	16	<0.5	4	<1
6	RNs-5		<20	1024	1024	2	4	<1
6	RTs-5		403	<1	<1	<1	<1	<1
7	RNo-5	Challenge only	<20	1024	64	32	4	<1
7	ROs-5		<20	4	16	2	<1	<1
7	RSp-5		<20	1	16	16	1	4
7	Rko-5		<20	256	1024	64	6	1

1 - Neutralizing Ab titers against homologous SHIV-HXBT were tested in vaccinated animals on day of virus challenge.

2 – PBMC at each time point were tested by co-cultivation assay for virus production.

TABLE 4. Neutralizing antibody response and viral load with homologous SHIV-vpu$^+$ rechallenge

				Week 1		Week 2		Week 3		Week 5		Week 8	
		DNA	nAb	Proviral	Infectious	Proviral	Infectious	Proviral	Infectious	Proviral	Infectious	Proviral	Infectious
Animal #	Group	Vaccination	Titer[1]	DNA	Cells[2]	DNA	Cells	DNA	Cells	DNA	Cells	DNA	Cells
RMw-5	2	id + IL-12	15	-	<1	+	<1	-	<1	-	Nt	-	<1
RDw-5	4	g.g.	<20	-	1	+	1	+	4	+	nt	+	<1
RFw-5	5	g.g. + IL-12	1,559	-	<1	-	<1	-	<1	-	nt	-	<1
RGt-5	6	gp160	338	-	<1	-	<1	-	<1	-	nt	-	<1
RDt-5	6	gp160	2,880	+	16	+	1	+	1	+	nt	+	1
RTs-5	6	gp160	44	-	<1	-	<1	-	<1	-	nt	-	<1
RCo-5		None		nt	16	Nt	>1024	nt	nt	nt	>1024	nt	4
RFo-5		None		nt	1	Nt	>1024	nt	nt	nt	>1024	nt	<1
RUo-5		None		nt	16	Nt	>1024	nt	nt	nt	>1024	nt	4
RNq-5		None		nt	1	Nt	>1024	nt	nt	nt	>1024	nt	1

[1] Neutralizing antibody titer against SHIV-HXBT on day of SHIV-vpu$^+$ rechallenge.

[2] Number of infectious cells per 10^6 PBMC.

TABLE 5. Cell-associated viral load following challenge with SHIV 89.6P

Animal #	Group	DNA Vaccination	Neutralizing Ab Titer [1]	# Infectious Cells per 10^6 PBMC					
				Week 1	Week 2	Week 4	Week 8	Week 12	Week 24
RMw-5	2	id + IL-12	<20	<1	64	4	1	1	<1
RDw-5	6	Genegun	<20	<1	64	<1	<1	<1	<1
RFw-5	7	Genegun + IL-12	<20	64	>1024	256	256	256	<1
RGt-5	8	gp160 alone	<20	64	>1024	16	4	4	4
RDt-5	8	gp160 alone	<20	1	1	4	1	1	1
RTs-5	8	gp160 alone	<20	64	>1024	64	64	64	64
RJq-5		None		256	>1024	256	64	64	16
RZq-5		None		64	>1024	256	256	256	64
RGr-5		None		64	>1024	256	1024	1024	64
RTr-5		None		256	>1024	256	256	256	1024

1 – Serum was tested for neutralizing antibody activity against SHIV89.6P at time of challenge.

TABLE 6. Immune responses following challenge with SHIV89.6P

			Proliferative Responses[2]										
	Neutralizing Ab Titer[1]		Expt 1 (Week 11)				Expt 2 (Week 15)				CTL Responses[3] (Week 8)		
Animal #	Week 8	Week 12	Nef	Gag	Env	Con A	Nef	Gag	Env	Con A	Gag-Pol	Nef	Env
RMw-5	243	4,969	6.7	4.4	7.9	652.4	2.1	7.4	2.5	142.5	8/12/11	-/-/-	4/9/14
RDw-5	<20	<20	1.1	11.2	8.5	74.9	2.1	5.8	6.8	63.2	18/13/10	-/-/-	-/-/-
RFw-5	282	9,030	4.2	2.3	19.3	281.4	nt	nt	nt	nt	-/-/-	-/-/-	-/-/-
RGt-5	307	12,580	nt	nt	nt	nt	0.8	2	0.9	35.1	nt	nt	nt
RDt-5	<20	<20	1.8	11.4	1.7	272.3	3	4.1	2.4	143.2	24/21/10	6/-/-	6/4/-
RTs-5	<20	<20	nt	nt	nt	nt	0.9	1.6	0.9	22.6	nt	nt	nt

[1] Recipricol serum titers from inoculated animals with neutralizing activity against SHIV89.6P.

[2] Proliferation stimulation indicies for PBMC in the presence of soluble antigens.

[3] Percent specific kiling of autologous BLCL expressing SIVmac239 *gag-pol*, SIVmac239 *nef* or HIV-IIIB *env* is shown for 3 different E:T ratios - For RMw-5: 117/39/13:1; RDw-5:173/58/19:1; RFw-5: 19/6/2:1; RDt-5: 100/33/11:1 nt - not tested

List of Personnel

DAMD17-94-J-4431

Name	Position	Dates	Dates	Months
TIM BABA	Assistant Professor	7/1/95 to 6/30/96		12
MIHAELA BAZALAKOVA	Summer student	7/1/96 to 7/31/96		1
MIGUEL COREN	Dishwasher	12/19/94 to 12/30/94		0.5
ANNE DISORBO	Administrative	8/12/96 to 10/31/97		14.5
ALICE FINK	Research Technician	4/1/97 to 11/30/97	4/1/98 to 5/8/98	9
CHRISTOPHER GALLEGOS	Administrative	10/1/98 to 9/29/99		12
LAURIE GERONIMO	Administrative	5/1/95 to 6/19/96		13.5
YUWEN HU	Research Technician	9/30/94 to 3/30/96		19
EILEEN IRAOLA	Dishwasher	8/14/95 to 9/1/95		0.5
YONG JEONG	Post Doc	11/1/94 to 1/31/95		3
JOSEPH HERNANDEZ	Summer student	7/7/97 to 8/29/97		2
ROBERT KLEIN	Summer student	6/10/96 to 8/31/96		2
MICHAEL LIM	Summer student	5/29/95 to 12/31/95		7
VLADIMIR LISKA	Instructor	4/1/95 to 12/31/96	4/1/97 to 3/31/98	33
CHARLES MCMILLEN	Dishwasher	9/30/94 to 8/19/96		23.5
ROBERT RASMUSSEN	Instructor	4/1/95 to 9/29/99		54
NANCY RAY	Post Doc	12/1/97 to 3/31/98		4
SUZANNE RESS	Administrative	11/1/94 to 5/10/95		7
NITALIYA ROZENVAYN	Summer student	6/9/97 to 7/25/98		13
RUTH RUPRECHT	Principal Investigator	9/30/94 to 9/30/97	7/1/98 to 8/31/99	50
GEORGE SERBAN	Summer student	7/5/95 to 9/30/95		2
SUSAN SHARP	Administrative	12/1/97 to 9/30/98	4/1/99 to 9/29/99	14
RYAN SWENERTON	Summer student	6/18/99 to 9/30/99		3
JOSEF VLASAK	Research Technician	6/1/98 to 9/29/99		16
YULAN WANG	Research Technician	6/22/98 to 9/29/99		15
LAURIE WHITE	Administrative	5/25/96 to 6/8/96		0.5
YVONNE WILLIAMS	Temporary	10/11/97 to 10/11/97		0.03